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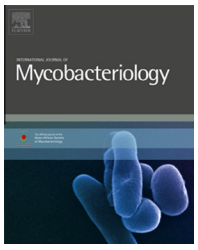

Isolation, identification and molecular characterization of *Mycobacterium bovis* from tuberculin-positive cattle in Hamedan province of Iran

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ABSTRACT

Introduction: Tuberculosis (TB) remains a major cause of death in many countries. Bovine tuberculosis caused by *Mycobacterium bovis* is one of the key members of the *Mycobacterium tuberculosis* complex. Current methods need long incubation times, which is in contrast to the necessity for rapid and accurate identification and isolation. Molecular techniques, such as RFLP fingerprinting, for accurate identification and differentiation of *M. tuberculosis* isolates are a more desirable approach.

Materials and methods: In a 12-month study plan, which began in November 2011, lymph nodes obtained from 92 samples of dairy cattle yielded tuberculin-positive marks that were collected from 10 different regions of the province; 43 isolates were identified by acid-fast culture. Using biochemical tests along with IS6110 specific fragment search, colonies were proved to belong to *M. tuberculosis* complex and more precisely *M. bovis* strains. PVUII enzymatic digestion of their DNA was followed by Southern blotting and hybridization with DR and PGRS markers. Finally, RFLP fragment hybridized with substrate BCIP and NBT was carried out, and their results were evaluated by GelproAnalyser software.

Results: All 43 isolates out of the 92 samples that were analyzed by RFLP with two probes of DR and PGRS, interestingly, proved the circulation of an identical strain of *M. bovis* throughout the whole province.

Discussion and conclusion: Assessing the genomic patterns of all the strains and comparing the results with previous studies conducted in the area revealed that, while they represent the circulation of a common ancestral clone, as a preliminary successful achievement in industrial and semi-industrial dairy farms located in the zone of the tuberculosis control program, they still have a long way to go to meet the ultimate eradication goals.

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